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# Vesicles Accelerate Proton Transfer from Carbon up to 850-fold

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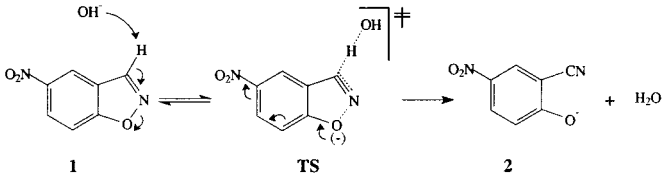
## ABSTRACT




**We have analyzed the different catalytic effects of surfactant aggregates upon the rate-determining hydroxide ion induced deprotonation reaction of 1. Vesicles are more effective catalysts than micelles, most likely providing a more apolar microenvironment at the substrate binding sites. We suggest that this leads to a catalytic reaction involving less strongly hydrated hydroxide ions. In the case of DODAB and DODAC vesicles, binding of cholesterol to the bilayer further increases the catalytic efficiency.**

Ever since the thorough mechanistic characterization of the elimination reaction **1** → **2** by Kemp,[2] this model reaction for the important proton transfer from carbon has been a prime target for enzyme mimics. Guided by Kemp's imperatives on how to bring about catalysis, a number of catalytic systems have been developed.[3] Most notable is a large solvent effect, in particular for anionic bases, where the rate differences between aprotic and protic solvents amount to up to $10^8$. Our objective is to find out whether it is possible to employ surfactant aggregates as simple enzyme mimics for this reaction. In addition, the results could help in understanding (artificial) enzyme efficiency in terms of desolvation, entropy factors, etc.

Since the Kemp elimination catalyzed by anionic bases is especially solvent sensitive, we looked at aggregate catalysis at a high pH value (11.35, [NaOH] = 2.25 mM) where turnover is expected to be dominated by the hydroxide-catalyzed reaction. The reaction rate for the elimination is considerably increased (up to 400 times) by the presence of cationic micelles formed from $C_{12}$PyrI, DTAB, CTAB, CTACl, and OTACl[4] with regard to the reaction in water. The typical biphasic pattern is observed (Figure 1).[5] In a simple model the concentration of reactive hydroxide ions in the micellar Stern layer increases up to a maximal value. At still higher surfactant concentrations unreactive counterions lead to a rate decrease as they replace hydroxide ions, thereby reducing the available species of increased reactivity. This experimental behavior can be explained quantitatively on the basis of Romsted's pseudophase model.[6]

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(4) $C_{12}$PyrI, 1-methyl-4-dodecylpyridium iodide; DTAB ($C_{12}3CN^+Br^-$), dodecyltrimethylammonium bromide; CTAB ($C_{16}3CN^+Br^-$), cetyltrimethylammonium bromide; CTACl ($C_{16}3CN^+Cl^-$), cetyltrimethylammonium chloride; and OTACl ($C_{18}3CN^+Cl^-$), octadecyltrimethylammonium chloride.

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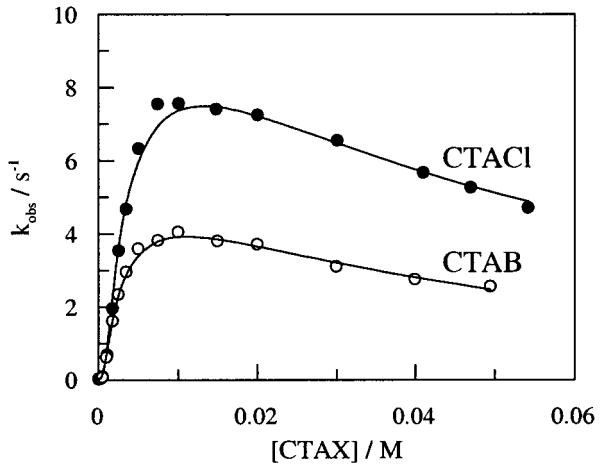


**Figure 1.** Influence of surfactant concentration on the alkaline decomposition of **1**. (○) CTAB and (•) CTACl. [NaOH] = 2.25 mM (pH = 11.35). [**1**] = $2 \times 10^{-5}$ M. The lines represent the best fit to the pseudophase model.

Comparing the reactivities in the micellar pseudophase with the corresponding reactivity in bulk water ($k_m^2/k_w^2$, see Table 1), the $k_m^2$ values are 3.6−6.6 times larger than the corresponding value in bulk water (a situation usually not encountered for nucleophiles).[7] This can be attributed, at least partly, to a medium effect. In fact, the Stern layer has a dielectric constant of ca. 35,[8] markedly lower than that of water. The influence of the dielectric constant upon the reaction rate was investigated by studying the elimination reaction in ethanol. The bimolecular rate constant for the reaction ($k_w^2 = 14.69\ M^{-1}\ s^{-1}$, $k_{ethanol}^2 = 6711 \pm 65\ M^{-1}\ s^{-1}$) shows that a decrease of the polarity of the solvent results in an increase in reaction rate. Therefore, we contend that the catalytic effects observed are due to the increase of the local reagent concentrations in the Stern layer and to the lower dielectric constant resulting in hydroxide ions that are less strongly hydrated.[9] The catalytic efficiency of the different micelles ($k_{max}/k_w$, Table 1) increases with increasing the chain length (47:117 for DTAB:CTAB and 230:413 for CTACl:OTACl); this trend corresponds to the higher value of the binding constant of **1** to the micelle, $K_{ass}$. It is also interesting to point out the different catalytic effects for micelles with the same chain length but with different counterions (117:230 for CTAB and CTACl, respectively); this trend is due to the different ability of the counterions to be replaced by $OH^-$ ions.[5]

**Table 1.** Kinetic Parameters for the Alkaline Decomposition of **1** in the Presence of Different Micelles Analyzed in Terms of the Pseudophase Model

| Surf | [1]$K_{ass}/M^{-1}$ | [2] $k_m^2/M^{-1}\ s^{-1}$ | [3] $k_m^2/k_w^2$ | [4]$k_{max}/k_w$ |
|---|---|---|---|---|
| $C_{12}$Pyr-I | [5] 27±1 | 53.5 | 3.6 | 48 |
| DTAB | [5] 28±1 | 81.2 | 5.5 | 47 |
| CTAB | [6] 43±1 | 50.3 | 3.4 | 117 |
| CTACl | [6] 41±2 | 79.1 | 5.4 | 230 |
| OTACl | [6] 64±5 | 97.6 | 6.6 | 413 |

[1] Binding constant of the substrate to the micelle. [2] Bimolecular rate constant in the micelle. [3] $k_w^2$ =14.69 $M^{-1}\ s^{-1}$ in water at 25°C; Ratio of micellar / water bimolecular rate constants. [4] Ratio of the maximum observed rate constant / rate constant in the absence of surfactant at the same pH. [5] [NaOH]= 1.60 mM (pH= 11.20). [6] [NaOH]= 2.25 mM (pH= 11.35). T= 25°C.

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We have studied the effects of different vesicle-forming surfactants: DDAB, DODAB, and DODAC[10] at a constant NaOH concentration (2.25 mM, pH = 11.35). Figure 2 shows

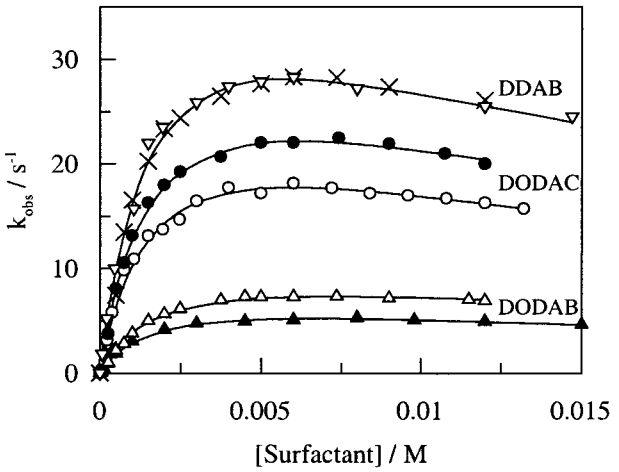


**Figure 2.** Influence of surfactant concentration on the alkaline decomposition of **1** in the absence of cholesterol, (▽) DDAB, (○) DODAC, and (▲) DODAB, and in the presence of 10.0% (w/w) cholesterol, (X) DDAB, (Δ) DODAB, and (•) DODAC (6.6% (w/w)). [NaOH] = 2.25 mM (pH = 11.35). [**1**] = $2 \times 10^{-5}$ M. The lines represent the best fit to the pseudophase model.

that the reaction rate increases to a maximum with increasing concentration of the different vesicles, after which a slight decrease in $k_{obs}$ is observed. The $k_{max}/k_w$ values have magnitudes up to 850 (Table 2) and show the high catalytic efficiencies of vesicular bilayers.

Kinetic data can again be quantitatively analyzed using the pseudophase ion-exchange model.[6] As with the cationic micelles, the cationic nature of the surfactants favors the presence of $OH^-$ ions in the vesicular pseudophase and the overall reaction rate is equal to the sum of the rates in the vesicular and aqueous pseudophases.

In applying the pseudophase model (Table 2 summarizes the kinetic data), we assumed a single $k_{ves}$ value corresponding to the rate-determining deprotonation at the inner and outer sides of the vesicle bilayer and a fast equilibrium of

**Table 2.** Comparison of the Catalytic Efficiency of the Vesicles in the Presence and in the Absence of Cholesterol

| Surf | [1] $D_H$/nm | % wt Cholesterol | [2] $K_{ass}$ / $M^{-1}$ | [3] $k^2_{ves}$ /$M^{-1}$ $s^{-1}$ | [4] $k^2_{ves}$ (C)/ $k^2_{ves}$ (no C) | [5] $k^2_{ves}$ / $k^2_w$ | [6] $k_{max}$ / $k_w$ |
|---|---|---|---|---|---|---|---|
| DDAB | 32 | 0 | 78±5 | 277.1 | 1.0 | 18.5 | 858 |
| | 27 | 10.0 | 78±5 | 277.1 | | 18.5 | 858 |
| DODAB | 37 | 0 | 61±3 | 63.2 | 1.6 | 4.3 | 161 |
| | 47 | 10.0 | 51±5 | 99.8 | | 6.8 | 223 |
| DODAC | 40 | 0 | 67±5 | 183.1 | 1.4 | 12.5 | 550 |
| | 42 | 6.6 | 64±3 | 251.4 | | 17.1 | 674 |

[1] Hydrodynamic diameter. [2] Binding constant. [3] Bimolecular rate constant in the vesicle. [4] Ratio between vesicular rate constants in the presence and in the absence of cholesterol. [5] Ratio of vesicular / aqueous bimolecular rate constants. [6] Ratio between the maximum observed rate constants in the presence and in the absence of surfactant at the same pH (11.35, [NaOH]= 2.25 mM). T= 25°C.

the substrate for binding at both reaction sites. The assumption of a single rate constant is reasonable, taking into account that no two-phase kinetics were found.[11] Moreover, the outer area in small vesicles corresponds to ca. 70% of the total available reaction area, and it is expected that the major site of reaction occurs at the outer surface.

We have calculated the bimolecular rate constant in the bilayer, $k^2_{ves}$. In all the vesicles $k^2_{ves}$ is higher than the rate constant in water, most likely due to the reduced micropolarity at the vesicular pseudophase. The $k^2_{ves}$ values are higher than those obtained for the micelles ($k^2_m$), compare Tables 1 and 2, which is consistent with the lower micropolarity at the vesicular surface relative to that at the micellar surface.[12] We note that the different catalytic effects for vesicles with different chain lengths ($k_{max}/k_w$), at least for DDAB and DODAC vesicles, are larger than those for the reactions in the presence of micelles.

The catalytic efficiencies of the vesicles at 25 °C follow the order DDAB > DODAC > DODAB ($k_{max}/k_w$; 860:550:160, respectively). Initially we anticipated a higher rate enhancement with an increasing chain length and an accompanying lower micropolarity at the vesicular binding sites. However, the phase transition temperatures[10] of the respective bilayers indicate that the DODAB and DODAC bilayers are in the rigid state at the reaction temperature and that the DDAB bilayer is in the fluid (liquid crystalline) state. Therefore, the relative inefficiency of bilayers with two $C_{18}$ chains is attributable to membrane rigidity. This peculiar catalytic effect varying with the ammonium bilayer membrane rigidity has been observed previously.[13]

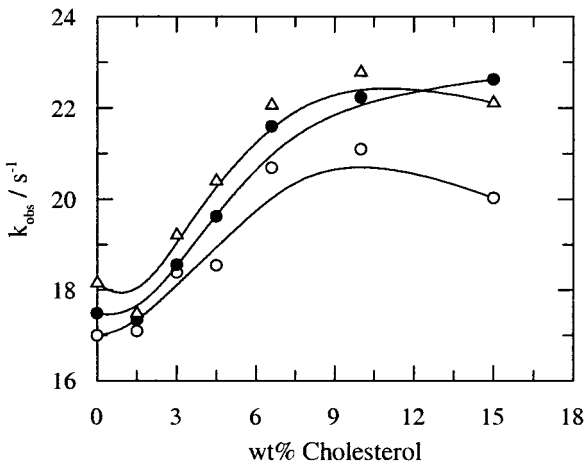


**Figure 3.** Influence of cholesterol on the overall reaction rate at three different DODAC concentrations: (O) 3.75 mM, (•) 4.50 mM, and (Δ) 6.00 mM. [NaOH] = 2.25 mM (pH = 11.35). [**1**] = 2 × $10^{-5}$ M. The lines are merely to guide the eye.

We note the different catalytic effects for vesicles with the same chain length but different counterions, DODAB and DODAC ($k_{max}/k_w$; 160:550, respectively). Small amounts of NaBr (at constant DODAC concentration) decrease the rate of the deprotonation reaction considerably near its

(9) Consistent with this interpretation, $k^2_{OH^-}$ is enhanced by a factor of 4400 going from water to 75 vol % DMSO−$H_2O$.

(10) DDAB ($2C_{12}2CN^+Br^-$), didodecyldimethylammonium bromide; DODAB ($2C_{18}2CN^+Br^-$), dioctadecyldimethylammonium bromide; and DODAC ($2C_{18}2CN^+Cl^-$), dioctadecyldimethylammonium chloride. Vesicles have been prepared by the sonication methods (bath, probe). Typically, the preparation consisted of the sonic dispersion (0.015 M) in 10 mL of water using a Branson 250 sonicator or an ultrasonic bath (Lab. Supplies Co, Inc.; model G112SP1T, 600 V, 80KC, 0.5 V) at an average temperature of 55 °C, which is above the phase transition temperatures of the different vesicles; 12 °C (DDAB), 34−36 °C (DODAB), 36 °C (DODAC) taken from Okahata, Y.; Ando, R.; Kunitake, T. *Ber. Bunsen-Ges. Phys. Chem.* **1981**, *85*, 789 and references therein. We checked out the possibility of vesicle fusion (increase of turbidity) following the reaction at 250 nm. In all the cases, we did not observe any change in the absorbance.

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maximum. Addition of 0.50 mM NaBr leads to a decrease in the reaction rate from 12.9 to 7.60 $s^{-1}$.[14]

We also studied the influence of cholesterol upon the deprotonation reaction in the presence of vesicles formed from DDAB, DODAB, and DODAC (Figure 3). Except for DDAB vesicles, the catalytic efficiency is higher than that in the absence of cholesterol (Table 2).

Because of the presence of cholesterol, the bilayer initially becomes less rigid and the phase transition temperature is lower (33.0 °C with 17.2 wt % of cholesterol) and the transition range is wider.[15] But addition of cholesterol also leads to a loss of counterion binding to the bilayer, which becomes more nonpolar and lyophobic.[16] Consequently we have two opposite effects, the lower concentration of the reactive $OH^-$ ions leading to rate retardation and less polar vesicular binding sites that increase the reaction rate. In accord with this rationalization, we find that at a constant DODAC concentration close to the maximum reaction rate (Figure 3), an increase in the binding of cholesterol results in an increase in the observed rate constant.

In summary, we find that surfactant aggregates efficiently catalyze the rate-determining deprotonation reaction of **1**. The catalytic effects are reminiscent of those observed for the unimolecular decarboxylation of 6-nitrobenzisoxazole-3-carboxylate.[13c] Vesicles are more effective than micelles, most likely providing a less polar microenvironment at the substrate binding sites for the deprotonation of **1**. This probably leads to a catalytic reaction involving less strongly hydrated hydroxide ions, resulting in overall rate enhancements up to a factor of 850. In the case of DODAB and DODAC vesicles, binding of cholesterol to the bilayer further increases the catalytic efficiency.

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(14) [DODAC] = 2 mM, [NaOH] = 2.25 mM (pH = 11.35), $T$ = 25 °C.

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